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ASSORTATIVE MATING IN INSECTS: SEXUAL SELECTION VS. SAMPLING ARTIFACT

Ruth Bernstein and Stephen Bernstein

ABSTRACT.—In this report we analyze patterns of size-assortative mating in an insect genus by (1) describing the consistency and strength of assortative mating among populations of a species and among species of a genus, and (2) determining the role of sampling artifacts in generating this nonrandom pattern of mating. Our data consist of 18 samples of soldier beetles (8 species of the genus Chauliognathus) from 17 sites within the southwestern United States. Of the 18 samples, just 6 exhibited significant correlations between elytron lengths of mating pairs. We show how these and other significant correlations are generated by 3 kinds of sampling artifacts: mixed-species samples, mixed-population samples, and mixed-microsite samples.

Key words: assortative mating, sampling, sexual selection, soldier beetles, spatial covariation, Chauliognathus.

Body size plays a crucial role in most aspects of an insect’s ecology, and so we expect natural selection to optimize body size in relation to the resources and microclimate of an insect population. Natural selection for optimal size in relation to ecology may be constrained, however, by sexual selection when relative body size influences mating success. Sexual selection for larger body size can generate a pattern of positive assortative mating (Fairbairn 1988, Crespi 1989, Arnquist et al. 1996) when one or both of its components—mate choice and intrasexual competition for mates—favor larger body sizes (Andersson 1994).

Mate choice in insects is seen most often as a preference by males for larger females, probably because larger females carry more (and sometimes larger) eggs (Ridley 1983, Reiss 1989, Honek 1993, Kim 1997, Cueva del Castillo et al. 1999, Hirschberger 1999), but also, in some species, because larger females provide better maternal care (Kim 1997, Strohm and Linsenmair 1997). Mate choice by females is less clear (Dugatkin and Godin 1998), due to the difficulty in isolating this behavior from the results of male-male contests for access to females. If there is female choice, it may favor larger males because they provide more nutrients (Gwynne 1982, 1988, Brown 1990a), better paternal care (Birkhead 2000), or better sons in terms of attracting females (Fisher 1930).

Intrasexual competition in insects is typically confined to males because winners of this competition can fertilize the eggs of many females. Larger males may have an advantage in contests with other males (Blum and Blum 1979, Ridley 1983, Thornhill and Alcock 1983, Alcock 1995, Cueva del Castillo et al. 1999), in interacting with females (Tammaru et al. 1996), or in assuring that their sperm fertilize the eggs after insemination (Mason 1980, Thornhill and Alcock 1983).

Sexual selection generates positive assortative mating in 2 ways: (1) mate choice in which both males and females prefer larger mates and (2) mate choice by males only (a preference for larger females) combined with intrasexual competition among males, in which larger males have an advantage (Ridley 1983).

An observed pattern of assortative mating does not, however, necessarily imply sexual selection. A correlation between body sizes of mates can also develop from covariation in body sizes (Crespi 1989), in which the sizes of both males and females vary in the same way through time or space. Thus, a sample consisting of pairs that initiated copulation at different times during the season or at different places may show a pattern of assortative mating even though individuals are mating at random with regard to the sizes of potential mates they encounter. Both temporal covariation and
spatial covariation are sampling artifacts rather than forms of sexual selection. Whenever assortative mating is observed, it is prudent to rule out sampling artifacts before initiating studies of mate choice and intrasexual competition.

A puzzling aspect of size-assortative mating in insects is its inconsistency—present in some species but not in others, closely related species; present in some populations of a species but not in others; present in a population during one year and not the next; present in a population during part but not all of the mating season (Mason 1972, Fairbairn 1988, Crespi 1989, Brown 1990b, Arquist et al. 1996, Bernstein and Bernstein 1998, 1999). Can some of this inconsistency be explained by sampling artifacts? The goal of the study reported here was to document the variability in size-assortative mating among populations of an insect species and among closely related species of an insect genus, and then to analyze the role of sampling artifacts, especially spatial covariation, in generating this nonrandom pattern. We chose soldier beetles (genus Chauliognathus Hentz) in the southwestern United States as our study animals for several reasons. First, we would be able to sample many populations of a species (the host plants have patchy distributions within wetter regions of the desert) and many closely related species (at least 18 species of the genus occur in the region). Second, sexual selection is likely to occur in soldier beetles because they are polygamous (Mason 1980, personal observations on marked individuals) with prolonged copulation (in a preliminary study with marked pairs, we found that 68% of mating pairs remained together for more than 5 hours and 34% for more than 17 hours). Moreover, a population of soldier beetles is likely to exhibit spatial covariation in body sizes because a female lays a single clutch (of approximately 19–70 eggs) and the predaceous larvae develop underground at the oviposition site (Robertson 1961, Woodhead 1981). Thus, adult beetles that emerge from a particular microsite are likely to be more similar, due to both genetic and environmental reasons, than adults collected from different microsites.

METHODS

We collected 18 samples of mating pairs of soldier beetles (Chauliognathus) from 17 sites in the southwestern United States. Each collection site encompassed an area <0.5 ha. Each sample was collected early on a single morning, when the beetles were too cool for rapid locomotion and so were likely to have been coupled since at least the previous evening. The beetles were preserved in 70% alcohol within a few hours of capture. We analyzed only pairs in which the aedeagus (male copulatory organ) was firmly held within the female genitalia. Males were identified to species by the shape of the aedeagus, using the key provided by Fender (1964). In some cases we identified females by species-specific correlations between length of the posterior elytron spot and length of the elytron (Bernstein and Bernstein 1998); in other cases they were assumed to be of the same species as their mates. The right elytron of each beetle was severed from the body and its maximum length measured to the nearest 0.01 mm, using a binocular microscope with an eyepiece micrometer.

The Pearson product correlation coefficient \( r \) of the relationship between elytron lengths of mating pairs was our test of assortative mating. We analyzed complete samples and subsets of samples, using a 0.05 level of significance and the more conservative 2-tailed test. While a complete sample is larger than each of its subsets, this difference affects only the level of significance; sample size has no effect on the numerical value of the correlation coefficient itself. Thus, our main concern here is with the shape of the cluster of points—to what degree the sampling artifact increases the linearity of the cluster, as measured by the \( r \) value. We provide levels of significance only to show how often a flawed sample could be interpreted to exhibit a nonrandom mating pattern.

RESULTS

Correlations between elytron lengths of mated pairs for all the samples are listed in Table 1. They vary greatly, not only among the 8 species of the genus but also among populations of the same species. For example, the \( r \) values for 5 populations of C. arizonensis are \(-0.05, 0.28, -0.14, 0.25, \) and \(0.04\); those for 6 populations of C. scutellaris are \(-0.11, 0.75, -0.06, 0.18, 0.38, \) and \(0.07\).

Surprisingly, 10 of 17 collection sites contained more than 1 species (9 sites had 2 species and 1 site had 3 species). In some cases the
Table 1. Samples of *Chauliognathus* Hentz: location, species in the sample, number of mated pairs, and correlation between elytron lengths of mated pairs.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>Species</th>
<th># pairs</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lingle, Wyoming</td>
<td><em>C. basalis</em> LeConte</td>
<td>51</td>
<td>0.18</td>
</tr>
<tr>
<td>2</td>
<td>Wiggins, Colorado</td>
<td><em>C. basalis</em></td>
<td>21</td>
<td>0.15</td>
</tr>
<tr>
<td>3</td>
<td>St. Vrain Canyon (lower), Colorado</td>
<td><em>C. deceptus</em> Fender</td>
<td>30</td>
<td>0.20</td>
</tr>
<tr>
<td>4</td>
<td>St. Vrain Canyon (middle), Colorado</td>
<td><em>C. basalis</em> and <em>C. deceptus</em>†</td>
<td>50</td>
<td>0.44**</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. basalis</em> (subset of sample 4)</td>
<td>19</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. deceptus</em> (subset of sample 4)</td>
<td>31</td>
<td>0.03</td>
</tr>
<tr>
<td>5</td>
<td>St. Vrain Canyon (middle, early)</td>
<td><em>C. deceptus</em></td>
<td>95</td>
<td>0.21*</td>
</tr>
<tr>
<td>6</td>
<td>Eldorado Springs, Colorado</td>
<td><em>C. basalis</em></td>
<td>40</td>
<td>0.15</td>
</tr>
<tr>
<td>7</td>
<td>Rocky Flats, Colorado</td>
<td><em>C. basalis</em></td>
<td>80</td>
<td>0.07</td>
</tr>
<tr>
<td>8</td>
<td>Cedar Crest, New Mexico</td>
<td><em>C. basalis</em></td>
<td>50</td>
<td>-0.01</td>
</tr>
<tr>
<td>9</td>
<td>Buckhorn, New Mexico</td>
<td><em>C. lewisi</em> LeConte (subset of sample 9)</td>
<td>44</td>
<td>-0.09</td>
</tr>
<tr>
<td>10</td>
<td>Lordsburg, New Mexico</td>
<td><em>C. werneri</em> Fender (subset of sample 9)</td>
<td>15</td>
<td>0.42</td>
</tr>
<tr>
<td>11</td>
<td>Deming, New Mexico</td>
<td><em>C. arizonensis</em> Fender and <em>C. scutellaris</em>†</td>
<td>45</td>
<td>-0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. arizonensis</em> (subset of sample 11)</td>
<td>30</td>
<td>-0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. scutellaris</em> (subset of sample 11)</td>
<td>15</td>
<td>0.75**</td>
</tr>
<tr>
<td>12</td>
<td>Hatchita, New Mexico</td>
<td><em>C. arizonensis</em> and <em>C. scutellaris</em>†</td>
<td>45</td>
<td>0.56***</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. arizonensis</em> (subset of sample 12)</td>
<td>15</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. scutellaris</em> (subset of sample 12)</td>
<td>30</td>
<td>-0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. lewisi</em> (subset of sample 12)</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>Tombstone, Arizona</td>
<td><em>C. obscurus</em> Schaeffer (subset of sample 13)</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>Douglas, Arizona</td>
<td><em>C. scutellaris</em> (subset of sample 13)</td>
<td>40</td>
<td>0.18</td>
</tr>
<tr>
<td>15</td>
<td>Marfa, Texas</td>
<td><em>C. obscurus</em> (subset of sample 14)</td>
<td>17</td>
<td>0.02</td>
</tr>
<tr>
<td>16</td>
<td>South of Marfa, Texas</td>
<td><em>C. scutellaris</em> (subset of sample 14)</td>
<td>26</td>
<td>0.38</td>
</tr>
<tr>
<td>17</td>
<td>Alpine, Texas</td>
<td><em>C. arizonensis</em></td>
<td>50</td>
<td>-0.14</td>
</tr>
<tr>
<td>18</td>
<td>Fort Davis, Texas</td>
<td><em>C. arizonensis</em></td>
<td>52</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*P < 0.05
**P < 0.01
***P < 0.001
†very similar species

External morphologies of coexisting species were so similar that we did not realize the sample consisted of more than 1 species until all the aedeagae were closely examined and checked with reference specimens. Because cryptic species often coexist, we included the mixed-species sample as a form of sampling artifact. Our results are organized into 3 kinds of sampling artifacts: mixed-species samples, mixed-population samples, and mixed-microsite samples.

**Mixed-species Samples**

We had difficulty distinguishing *C. arizonensis* from *C. scutellaris*, and *C. basalis* from *C. deceptus*. This type of sampling artifact accounts for 4 of the 6 significant correlations between body sizes of mating pairs in our 18 samples.

Mating pairs of *C. arizonensis* and of *C. scutellaris* were collected from the same blossoms of the host plant at 3 of our collection sites (samples 11, 12, and 16). In each case the combined sample showed assortative mating. We describe here sample 12, collected near Hatchita, New Mexico (elevation 1380 m). A scatterplot of elytron lengths of the mating pairs in this sample is shown in Figure 1(a).

Within each species the mating pairs show no assortative mating—for *C. arizonensis*, n = 15 and r = 0.28 (not significant); for *C. scutellaris*, n = 30 and r = -0.06 (not significant). When the sample includes both species (n = 45), the correlation coefficient increases to 0.56 (P < 0.001). A pattern of assortative mating emerges in the complete sample because, as can be seen in the scatterplot, males and
females of *C. scutellaris* are larger than males and females of *C. arizonensis*.

Another pair of cryptic species, *C. deceptus* and *C. basalis*, coexist at the same collection site and on the same blossoms of the host plant in St. Vrain Canyon near Lyons, Colorado (sample 4). A scatterplot of the elytron lengths of mating pairs in this sample is shown in Figure 1(b). Within each species there is no assortative mating—for *C. basalis*, $n = 19$ and $r =$
0.12 (not significant) and for C. deceptus, $n = 31$ and $r = 0.03$ (not significant). When both species are included ($n = 50$), the correlation coefficient increases to 0.44 ($P < 0.01$). The complete sample exhibits assortative mating because, as can be seen in the scatterplot, individuals (especially females) of C. basalis tend to be larger than those of C. deceptus.

Mixed-population Samples
As the size of a collection site increases, it may come to include more than 1 population of the same species. While we do not know whether any of our 4 samples contains more than a single population, we describe here what happens when samples of the same species from different collecting sites are combined.
Our 1st example is of 2 samples of *C. arizonensis*, sample 16 from a site just south of Marfa, Texas (elevation 1400 m), and sample 17 from Alpine, Texas (elevation 1340 m). The 2 sites are within 60 km of each other. A scatterplot of the elytron lengths of mating pairs is shown in Figure 2(a). Considering each sample by itself, the correlation coefficient for the south-of-Marfa sample (n = 27) is 0.25 (not significant) and for the Alpine sample (n = 52) it is 0.04 (not significant). When the 2 samples are combined, the correlation coefficient rises to 0.39 (P < 0.001). The higher correlation coefficient in the combined sample occurs because, as shown in the scatterplot, individuals (especially males) in the Alpine sample are larger than individuals in the south-of-Marfa sample.

The 2nd example is 2 samples of *C. deceptus*, sample 3 from a lower section of St. Vrain Canyon (elevation 1500 m) and sample 4 from a middle section of the canyon (elevation 1830 m). The 2 sites are within 10 km of each other. A scatterplot of the elytron lengths of mating pairs is shown in Figure 2(b). As single samples, the correlations between elytron lengths of mating pairs are low: for the lower-canyon site, n = 30 and r = 0.20 (not significant); for the mid-canyon site, n = 31 and r = 0.03 (not significant). Combined, the 2 samples have a correlation coefficient of 0.43 (P < 0.001). As the scatterplot indicates, the correlation increases because males and females in the mid-canyon sample are somewhat larger than those in the lower-canyon sample.

**Mixed-microsite Samples**

An early season sample of *C. deceptus* (sample 5) was collected from St. Vrain Canyon (elevation 1830 m) near Lyons, Colorado. We kept separate the mating pairs collected from each of 2 rabbitbrush (*Chrysothamnus nauseosus*) plants, positioned just 12 m apart. A scatter plot of this sample is shown in Figure 3. The correlation coefficients of mated pairs from plant A (n = 47 and r = 0.09) and from plant B (n = 48 and r = 0.07) are low and not significant, whereas that of the complete sample is higher (r = 0.21) and significant (P < 0.05). Assortative mating is observed in the complete sample because individuals from plant B (nearer a stream) are slightly larger than individuals from plant A.

**DISCUSSION**

We collected 18 samples of mating soldier beetles from various sites throughout the southwestern United States. Assortative mating, as defined by a significant correlation between elytron lengths of mating pairs, occurred in 6 samples. In 5 samples assortative mating is attributed to sampling artifacts—samples consisting (inadvertently) of more than 1 species

![Fig. 3. Correlation between elytron lengths of mating pairs in a mixed-microsite (2 different plants) sample of *C. deceptus*: r = 0.21; P < 0.05.](image-url)
and samples taken from more than 1 collecting site or more than 1 microsite within a collecting site. (The cause of assortative mating in the 6th sample is unknown).

It is remarkably easy to inadvertently collect a mixed-species sample of soldier beetles, as individuals of 2 or more species are often found mating at the same time on the same host plant (even on the same blossom), and some species pairs are extremely difficult to distinguish, even for an entomologist specializing in the genus. As stated by Miskimen (1966:14):

External morphological characteristics including color and color patterns are generally adequate for species identification of most specimens of Chauliognathini, but color variation, variable pronotal shape, and similar morphology among related species may make identification of some specimens difficult.

The single most important morphological feature (and, for some species, the only feature) used in determining species status is the aedeagus, or male copulatory organ (Fender 1964, Miskimen 1966). These organs are tiny, partly soft, and very similar among the more closely related species of the genus.

One is likely to inadvertently collect a sample from more than 1 population when attempting to obtain a large sample from a sparse population. It is generally not possible to know, when collecting insects, where the distribution of 1 population ends and another begins. We have shown in this report that combining samples from different collecting sites leads to a pattern of assortative mating, whereas in fact the insects are mating at random with regard to size of potential mates they encounter.

Assortative mating attributed to more than 1 microsite is our most interesting result, as it reveals a spatial mosaic of body sizes within a surprisingly small area—our 2 microsites were only 12 m apart. We segregated the pairs collected from the different microsites of this early season sample because we thought that spatial covariation of body sizes might exist when adults first emerge from underground where they developed as larvae. We found that beetles from each of 2 microsites were mating at random with regard to body size of potential mates they encountered, although beetles from the collecting site as a whole exhibited a pattern of assortative mating. We believe that spatial covariation at this site is an early season phenomenon for 2 reasons. First, soldier beetles are capable of dispersing throughout the ovule-shaped 0.5-ha site. Brown and Brown (1984) found that marked-and-released individuals dispersed a mean (+2 sE) distance of 15.5 ± 2.3 m (males) and 10.1 ± 3.3 m (females) within 24 hours. The mating season at our site continues for at least 4 weeks. Second, in a previous study at this site (Bernstein and Bernstein 1998, in which we ruled out temporal covariation as a sampling artifact), we found a decline in the correlation between elytron lengths as the mating season progressed. While we would have liked to follow the pattern of spatial covariation throughout the mating season in the study reported here, we could not do so because of the impact of the 1st sample on the density (and probably composition) of beetles at those microsites.

The samples collected in this study reveal a remarkable variability in assortative mating, as evidenced by the wide range of correlation coefficients among populations of a species and among closely related species. The higher r values occurred (with a single exception) in samples that contained 2 cryptic species. Other significant correlations can be attributed to spatial covariation among collecting sites (perhaps different populations) and among microsites within a collecting site. Spatial covariation as a mechanism for generating assortative mating has been largely unexplored in insects, although it has been reported in a crustacean (Birkhead and Clarkson 1980, Ward and Porter 1993, but see Dick and Elwood 1993). A spatial mosaic of body sizes may develop from an aggregated distribution of genotypes (e.g., clusters of siblings in a microsite or of gene pools among collecting sites) and/or an environment that is heterogeneous with regard to food, microclimate (Van Dijk and Den Boer 1992, Van Dijk 1994), density of competitors (Hirschberger 1998, 1999), or other factors that influence larval growth. Species with nondispersing larvae are likely to exhibit a spatial covariation of body sizes between the time of adult emergence and the time of adult dispersal. We conclude that at least some of the inconsistent patterns of assortative mating in insects may be explained by the following hypothesis: Spatial covariation of body sizes is present when adults first emerge from the microsites where they develop as larvae and then declines as dispersal mixes individuals from different microsites. The
strength of the correlation between body sizes of mating pairs depends, then, on the time during the mating season when the sample is collected.

**LITERATURE CITED**


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